Influence of Refeeding with Vitamin, Mineral and Fibre on Protein Synthesis and Messenger Ribonucleic Acid Content in the Liver and Muscle of Fasted Chicks

M. Aman Yaman, K. Kita[1], R. Pinontoan and J. Okumura
Laboratory of Animal Nutrition, Graduate School of Bioagricultural Sciences, Nagoya University
Nagoya 464-01, Japan

ABSTRACT : The influence of refeeding with either vitamin, mineral, fibre of water on protein synthesis and mRNA content in the liver and breast muscle of fasted chicks was investigated. At 15 d of age, chicks were fasted for 2 d and then refed either vitamin, mineral, fibre or water. The fractional synthesis rate (FSR) of protein was measured after 30 min of refeeding by using a large dose injection of L-2, 6[^{3}H] phenylalanine. In the liver, FSR was reduced by fasting and tended to increase but not significantly by refeeding with vitamin or mineral. FSR was not affected by refeeding with fibre or water. There was no influence of fasting and refeeding on ribosomal capacity (the RNA : protein ratio) and ribosomal efficiency (total protein synthesised per total RNA). The absolute synthesis rate (ASR) of liver protein and hepatic mRNA content were reduced by fasting and unchanged by refeeding. In the muscle, FSR, ASR and mRNA content were significantly decreased by fasting and not recovered by refeeding with either vitamin, mineral, fibre or water. It concluded that vitamin, mineral, fibre and water have little capacity to stimulate liver and muscle protein synthesis reduced by fasting.
(**Key Words**: Protein Synthesis, mRNA, Refeeding, Vitamin, Mineral, Fibre, Water, Fasted Chicks)

INTRODUCTION

It is well known that nutrient intake is an important factor influencing the rate of protein synthesis in animals. In rats, liver (McNurlan et al., 1979; Davis et al., 1993) and muscle (Garlick et al., 1983; Millward et al., 1983) protein synthesis was decreased by fasting and recovered by refeeding (Mosoni et al., 1996; Yoshizawa et al., 1995). In young chickens, the response of liver and muscle protein synthesis to fasting and refeeding was similar to that observed in mammalian species (Nieto et al., 1994; Kita et al., 1996a, b). These findings show that protein synthesis in growing animals is decreased under malnutritional conditions such as fasting, and in many cases, can be recovered by refeeding with various nutrients. Recently, it has been reported that tissue mRNA content was also affected by changes in nutritional conditions. Fasting decreased myosin mRNA level in human muscle (Blomqvist et al., 1991) and albumin mRNA level in the liver of rats (Yap et al., 1978). In chicks, total hepatic mRNA content was also reduced by fasting and recovered by refeeding (Kita et al., 1993a, b).

The influence of refeeding of various nutrients such as protein, carbohydrate and fat on protein synthesis and mRNA content have been studied in the liver and muscle of fasted chicks (Kita et al., 1996a, b). In these reports, it was revealed that fasting decreased protein synthesis in both tissues, and refeeding of either protein, carbohydrate or fat promptly stimulated an increase in liver and muscle protein synthesis. However, there is virtually no information available concerning the influence of other nutrients, such as vitamin, mineral or fibre, on tissue protein synthesis in chicks. Therefore, in the present study, we have examined the influence of refeeding with vitamin, mineral, fibre and water on protein synthesis and mRNA content in the liver and muscle of fasted chicks.

MATERIALS AND METHODS

Two hundred days old single-comb White Leghorn male chicks were obtained from a local hatchery (Hattori Yokei-en Ltd, Nagoya, Japan) and reared in a temperature-controlled room (29 ± 2℃) illuminated for 24 h. During this period, chicks fed *ad libitum* on a commercial starter diet (CP 21.5%, ME 12.1 kJ/g; Marubeni Siryou Ltd, Tokyo, Japan) until 7 d of age. At this age, 60 chicks with similar body weight were

[1] Address reprint requests to K. Kita.
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selected, distributed into 6 groups of 10 chicks and kept individually in stainless steel metabolism cages. Chicks were fed on a semi-purified control diet (CP 20% and ME 12.6 kJ/g) until 15 d of age. The composition of the diet is represented in table 1.

Table 1. Composition of experimental diets (g/kg)

Sources	Control	Vitamin	Mineral	Fibre
Isolated soybean protein	239.0	–	–	–
L-Methionine	2.9	–	–	–
L-Threonine	1.2	–	–	–
Glycine	4.2	–	–	–
Corn starch	302.0	–	–	–
Sucrose	200.0	–	–	–
Cellulose	157.7	–	–	1,000.0
Corn oil	30.0	–	–	–
Mineral mixture[1]	58.5	–	1,000.0	–
Vitamin mixture[2]	2.0	1,000.0	–	–
Choline chloride	1.5	–	–	–
Inositol	1.0	–	–	–
Refeeding level (g/chick)	4.8	0.01	0.28	0.77

[1] The mineral mixture supplied (per kg of diet): calcium phosphate (dibasic), 20.7 g; calcium carbonate, 14.8 g; potassium dihydrogenphosphate, 10.0 g; potassium chloride, 3.0 g; sodium chloride, 6.0 g; magnesium sulphate, 3.0 g; ferric sulphate, 500 mg; manganese sulphate, 350 mg; potassium iodide, 2.6 mg; copper sulphate, 40 mg; zinc oxide, 62 mg; cobalt chloride, 1.7 mg; sodium molybdate, 8.3 mg; sodium selenite, 400 μg (Kita and Okumura, 1993).

[2] The vitamin mixture supplied (per kg of diet); calcium pantothenate, 15 mg; riboflavin, 6 mg; pyridoxine hydrochloride, 4 mg; nicotinic acid, 40 mg, pteroylmonoglutamic acid, 1.5 mg; biotin, 200 μg; cyanocobalamin 20 μg; thiamine hydrochloride, 3 mg; retinyl acetate, 1 mg; cholecalciferol, 5 μg; thocopheryl acetate, 10 mg; menadione, 500 μg (Kita and Okumura, 1993).

Our previous findings indicated that the rate of muscle and liver protein synthesis was decreased significantly by 2-d fasting and was recovered by refeeding of experimental diets for 30 min (Kita et al., 1996a, b). In the present study, therefore, chicks were also fasted for 2 d and thereafter the rate of protein synthesis was measured at 30 min after refeeding of each nutrient. At 15 d of age, 50 chicks were fasted for 2 d and the remaining 10 chicks were allowed free access to the control diet. Chicks in each of 3 experimental groups (30 chicks in total) were force-fed only one of the experimental diets which contained either vitamins, minerals, or fibre which have been mixed with water. The amount of nutrients force-fed was equal to that of each nutrient in the control diet which was force-fed with water into the crop of chicks at one time (4.8 g). The amounts of each diet force-fed are shown in table 1. Water only was given to 10 fasted chicks and the remaining 10 fasted chicks were used as unfed controls. After 30 min of force-feeding, chicks were weighed and then treated to obtain liver and muscle protein synthesis measurements. Fractional synthesis rate (FSR) of liver and muscle protein was measured using a large dose injection (Garlick et al., 1980) of L-[2, 6-^{3}H] phenylalanine into a wing vein of chicks (1 ml/100 g body weight: 120 mM phenylalanine, 1.48 MBq/ml in 0.15 M sodium chloride). Two minutes after tracer injection (4 chicks) and 10 min after tracer injection (6 chicks) animals were killed by neck dislocation and thereafter breast muscle (*M. pectoralis superficialis*) and liver were quickly excised, washed with ice-cold physiological saline, blotted, weighed and immediately frozen in liquid nitrogen and than stored at −80℃ until analysis.

The chemical analysis for measuring FSR of liver and muscle protein was a modification of the method of Garlick et al. (1980), and was described previously (Kita and Okumura, 1993). Total RNA was isolated from approximate 1 g of tissue using acid-guanidine phenol chloroform method described by Chomczynski and Sacchi (1987). Total mRNA was purified from total RNA by poly (U) Sepharose 4B affinity chromatography (Grotha, 1976). The mRNA content was determined by measuring the absorbance at 260 nm. Detailed analytical methods were previously described by Kita et al. (1996a).

Data was analysed by one-way analysis of variance

(ANOVA) to test for overall differences among treatments. Tukey's studentized range test was used to determine significant differences between means. Statistical analyses were carried out using a commercial statistical package, SAS (1985).

RESULTS

Table 2 shows the influence of fasting followed by refeeding with either vitamin, mineral, fibre or water on body, liver and muscle weights of chicks. Body, liver and muscle weights of chicks were decreased by 2-d of fasting, and reduced weights were not recovered at 30 min after refeeding with either vitamin, mineral, fibre or water.

Table 2. Influence of refeeding with vitamin, mineral, fibre or water on body, muscle and liver weights of chicks after 2-d fasting

	Fed	Fasted	Refeeding after 2-d fasting				Pooled SEM
			Vitamin	Mineral	Fibre	Water	
Body weight (g)	148[a]	105[b]	106[b]	105[b]	105[b]	103[b]	1.8
Muscle weight (g)	5.48[a]	2.88[b]	3.21[b]	3.07[b]	3.15[b]	3.03[b]	0.2
Liver weight (g)	5.03[a]	3.23[c]	3.35[bc]	3.33[bc]	3.41[bc]	3.62[b]	0.1

[a,b,c] Means within the same raw with different superscript letters were significantly different at $p < 0.05$.

In the liver, protein, RNA and mRNA contents were significantly decreased by 2-d of fasting and were not reversed by any of the refeeding treatments (table 3). Liver FSR were reduced by 2-d of fasting and tended to increase but not significantly following refeeding with either vitamin or mineral. There was no influence of refeeding fibre or water only on FSR in the liver of fasted chicks. Fasting and refeeding did not change the ribosomal capacity (Cs; the RNA : protein ratio) or the ribosomal efficiency (K_{RNA}; total protein synthesised per total RNA) in the liver. The absolute synthesis rate (ASR) of livers was reduced by 2-d of fasting and was not affected by refeeding.

Table 3. Influence of refeeding with vitamin, mineral, fibre or water on protein, RNA and mRNA contents, ribosomal capacity for protein synthesis (C_S), the fractional (FSR) and absolute (ASR) protein synthesis rates, and ribosomal efficiency for protein synthesis (K_{RNA}) in the liver of fasted chicks

Parameters	Fed	Fasted	Refeeding after 2-d fasting				Pooled SEM
			Vitamin	Mineral	Fibre	Water	
Protein (mg)	1,090[a]	568[b]	602[b]	581[b]	580[b]	611[b]	48
RNA (mg)	39.3[a]	18.0[b]	18.6[b]	15.3[b]	18.7[b]	18.4[b]	3.5
mRNA (μg)	1,653[a]	837[b]	557[b]	712[b]	1,011[b]	701[b]	215
Cs (mg RNA : g protein)	37.2	32.0	30.8	27.0	32.6	30.3	4.1
FSR (%/d)	98.9[a]	63.2[b]	77.2[ab]	83.9[ab]	64.9[b]	68.0[b]	6.2
ASR (mg/d)	1,077[a]	345[b]	471[b]	487[b]	396[b]	419[b]	69
K_{RNA} (mg protein synthesised. mg $RNA^{-1} \cdot d^{-1}$)	33.8	18.5	25.1	32.0	22.0	22.8	4.7

In refed chicks, FSR was measured at 30 min after refeeding of experimental diets.
[a,b] Means within the same column with different superscript letters were significantly different at $p < 0.05$.
The number of chicks used was ten.

Table 4 shows the influence of fasting and refeeding with either vitamin, mineral, fibre or water on protein, mRNA and RNA contents, and other parameters relating to protein synthesis in the muscle of chicks. Protein, RNA and mRNA contents were significantly decreased by fasting and were not affected by refeeding with vitamin, mineral, fibre or water. As in the livers of chicks fasted for 2-d, muscle FSR was significantly decreased, and then

refeeding of each nutrient failed to stimulate FSR. There was no influence of fasting and refeeding on Cs in the muscle, whereas ASR and K_{RNA} were reduced by 2-d of fasting and remained so for 30 min after refeeding.

Table 4. Influence of refeeding with vitamin, mineral, fibre or water on protein, RNA and mRNA contents, ribosomal capacity for protein synthesis (C_S), the fractional (FSR) and absolute (ASR) protein synthesis rates, ribosomal efficiency for protein synthesis (K_{RNA}) in the muscle of food-deprived chicks

Parameters	Fed	Fasted	Refeeding after 2-d fasting				Pooled SEM
			Vitamin	Mineral	Fibre	Water	
Protein (mg)	1,115[a]	480[b]	544[b]	530[b]	529[b]	508[b]	48
RNA (mg)	10.4[a]	4.4[b]	5.0[b]	4.7[b]	4.7[b]	4.6[b]	0.3
mRNA (μg)	287[a]	77[b]	95[b]	118[b]	111[b]	106[b]	25
Cs (mg RNA : g protein)	9.4	9.3	9.4	8.9	9.0	9.0	0.5
FSR (%/d)	15.5[a]	8.5[b]	9.2[b]	10.3[b]	7.5[b]	10.3[b]	1.1
ASR (mg/d)	173[a]	40[b]	51[b]	53[b]	40[b]	55[b]	8.7
K_{RNA} (mg protein synthesised. mg $RNA^{-1} \cdot d^{-1}$)	16.6[a]	9.1[b]	10.7[b]	11.3[ab]	8.5[b]	11.6[ab]	1.3

In refed chicks, FSR was measured at 30 min after refeeding of experimental diets.
[ab] Means within the same raw with different superscript letters were significantly different at $p < 0.05$.
The number of chicks used was ten.

DISCUSSION

We have shown that liver and muscle FSR were markedly reduced by 2-d fasting and that values for FSR in both tissues of fasted chicks were 36 and 45% respectively of those found in fed chicks. Similar results have previously been reported for fasted chicks, which supports the data observed in the present study (Nieto et al., 1994; Kita et al., 1996a, b). Additionally, we have shown that 2-d fasting significantly reduced mRNA content in the liver and muscle, which is also in good agreement with our previous studies (Kita et al., 1993a, b).

There have been some reports that suggest an important role for both minerals and vitamins in maintaining liver and muscle protein synthesis. When rats received a zinc deficient diet for 24 d, muscle protein synthesis decreased significantly (Giugliano and Millward, 1987). A similar reduction in protein synthesis was observed in the livers of rats given a zinc deficient diet for 6 wk (Kimball et al., 1995). Dorup and Clausen (1989) also showed that protein synthesis was very sensitive to the level of dietary potassium. Also, Simard and Srivastava (1974) have reported that vitamin E caused a change in rabbit muscle protein synthesis. Tissue mRNA levels are also very sensitive to changes in dietary vitamin levels. For example, it has been reported that biotin deficiency decreased the amount of ornithine transcarbamylase mRNA in the liver of rats (Maeda et al., 1996). In the present study, however, when chicks were refed with either vitamin or mineral after 2-d of fasting, no significant increases in FSR and mRNA contents were observed in liver or muscle. This discrepancy concerning the influence of vitamin and mineral on protein synthesis may be a result of the difference in the period used for examining the influence of nutrients. In the previous reports, the influence of vitamin or mineral deficiency was examined for more than 3 wk. In contrast, changes in FSR and mRNA contents were measured at 30 min after refeeding in the present study. Therefore, the acute effects of vitamin and mineral on protein synthesis in the liver and muscle may be different after more prolonged refeeding. Another possibility is related to the contribution of other nutrients such as protein, carbohydrate and fat. Recently, we reported that decreases in liver and muscle FSR by fasting could be recovered by refeeding with either protein, carbohydrate or fat within 30 min after consumption (Kita et al., 1996a, b). Therefore, if these nutrients were available in the diet, vitamin and mineral might synergise with these components in stimulating protein synthesis.

It concluded that the refeeding of vitamin, mineral, fibre or water alone has little effect on protein synthesis and mRNA content of fasted chicks measure 30 min after refeeding.

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